Positive Regulatory Domain I (PRDM1) and IRF8/PU.1 Counter Regulate MHC Class II Transactivator (CIITA) Expression during Dendritic Cell Maturation

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Supplemental Figure Legends

Supplemental Figure 1: CIITA expression is silenced upon maturation of human myeloid DCs. Human lymphocyte-depleted PMBC were cultured in FCS-containing RPMI medium in the presence of GM-CSF (1000U/ml) and IL-4 (5ng/ml) for seven days. Maturation was induced with either LPS (10ng/ml) or macrophage-conditioned medium. **A)** Flow cytometry analysis of human DC cultures. Double stained cells were examined by two-color FACS and analyzed with FlowJO 6.0. CD11c staining is represented on the x-axis, while additional markers are represented on the Y-axis as indicated. B) Quantitative RT-PCR analysis of promoter specific CIITA isoforms and HLA-DRA mRNA expression. Amplification primers were designed within the unique Exon 1 for each of the three CIITA isoforms. Results are normalized to GAPDH expression plotted relative to the expression of the transcript in Day 3 immature DCs. Direct comparison of the expression levels has previously shown that CIITApI has the highest expression, CIITApIII has moderate expression and CIITApIV is extremely low (5). **C)** Multiple maturation stimuli suppress CIITA expression. Day 7 immature DCs were exposed to the stimuli indicated on the x-axis for 24 hrs. Stimuli include double-stranded RNA (200ng/ml), LPS (10 ng/ml), macrophage-conditioned media (50/50 v/v), TNFα (50ng/ml), IFNα (1000 U/ml). Data shown is the average of results obtained from 3 independent donors with standard deviation. Significance was determined using two-tailed, paired t-tests ($* \leq 0.05$).

Supplemental Figure 2: In vivo footprint of the human HLA-DRA promoter.
Immature DCs display an occupied promoter which is lost upon maturation. A) Upper strand of the HLA-DRA promoter is shown. Lane marked "ont" shows the complete guanine sequencing ladder from *in vitro* methylated DNA. All other lanes are in vivo methylated DNA samples. "Imm" represents immature DC in culture with GM-CSF and IL-4 for 7 days. "LPS" represent mature DC cultured with LPS (10 ng/ml) for 48 hours. All lane markings are as in Figure 1. B) Schematic of *in vivo* protein/DNA interactions at the HLA-DRA promoter. Protected and enhanced residues are indicated by the open and solid arrowheads, respectively. Known functional elements are indicated with boxes.

Supplemental Figure 3: CIITApI promoter homology between human and mouse. Aligned sequences for the proximal promoter region of human and mouse CIITApI is shown. Elements conserved between species are indicated with boxes which encompass both sequences will unique elements are only boxed on the relevant sequence. The transcription start is indicated for each promoter by the arrow and +1.

Supplemental Figure 4: **IRF8 transduction rescues MHC-II expression.** A) Bone marrow-derived murine *Irf8-/-* DCs were transduced with retroviral vectors encoding either IRF8-EGFP or EGFP-only as described (41). Two-color FACS analysis was performed using staining for CD11c and MHC Class II with live cell gates applied based on light scattering properties. Data shown are representative plots from several experiments. B) qRT-PCR analysis of CIITA mRNA isoforms. Data shown represent mean from three experiments +/- standard deviation.

Supplemental Figure 5: Analysis of activating factors. A) qRT-PCR analysis of mRNA for activating transcription factor induction during maturation of monocyte-derived DC. Data represent mean of 3 independent experiments +/- standard deviation, expressed as fold induction, relative to Day 3.. Immature DC were analyzed at day 3 (D3) and day 7 (D7). Maturation was induced by LPS (24h) or macrophage-

conditioned medium (48h) as indicated. B) Immunoblot analysis for activating transcription factors in immature and LPS-stimulated monocyte-derived DCs.

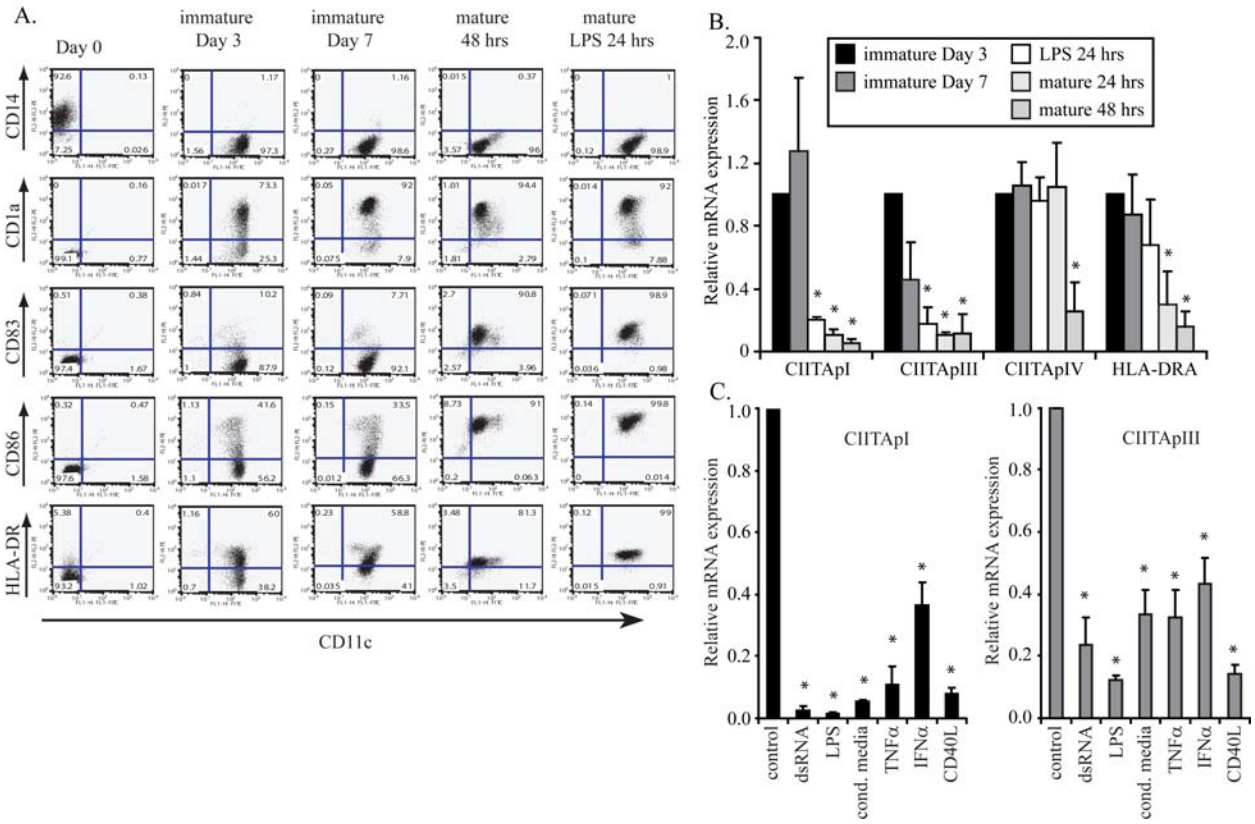
Supplemental Figure 6: PRDM1 is induced by maturation and co-localizes with G9a. A) Immunofluorescence staining of immature and mature human DCs. The antibodies used are indicated adjacent to each row. DNA was counterstained with DAPI. B) Co-localization of PRDM1 and G9a in mature human monocyte-derived DC (stimulated by LPS 10 ng/ml for 24 hrs). Upper row, co-immunostaining with anti-PRDM1 and anti-HLA-DR (green). Lower row, co-immunostaining with anti-PRDM1 (red) and anti-G9a (green). The merged confocal images indicate co-localization of PRDM1 and G9a.

Supplemental Figure 7: Mutational analysis demonstrates that upstream PU.1 element is not critical for PRDM1-mediated repression of human CIITApI promoter. A) Transient transfection of 293T cells with the CIITApI-p709 construct and the upstream Pu.1 mutants. Data was normalized to Renilla luciferase activity with the wildtype promoter activity set to 100%. Data shown represents the average of 3 experiments with error bars showing standard deviation. Fold repression is shown in the boxed text. Differences between PRDM1-mediated repression of each construct were not statistically significant ($p = .09$).

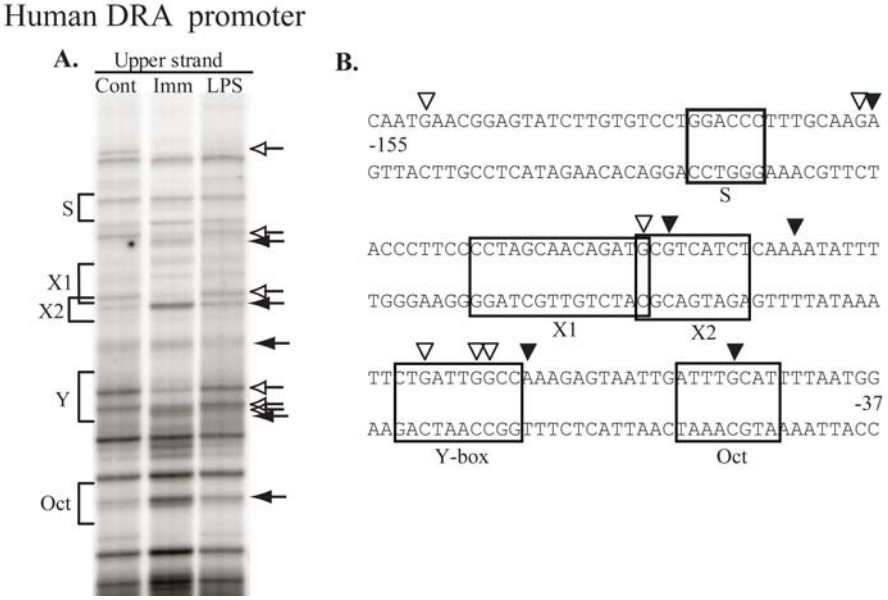
Supplemental Table 1: Oligonucleotide sequences utilized. Letter in red indicate mutated nucleotides.

Supplemental Figures

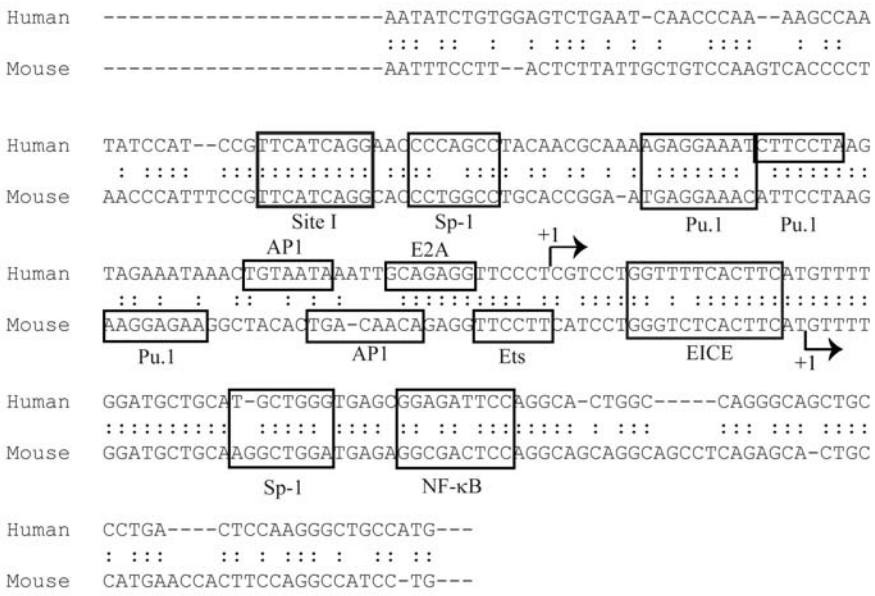
Supplemental Figure 1



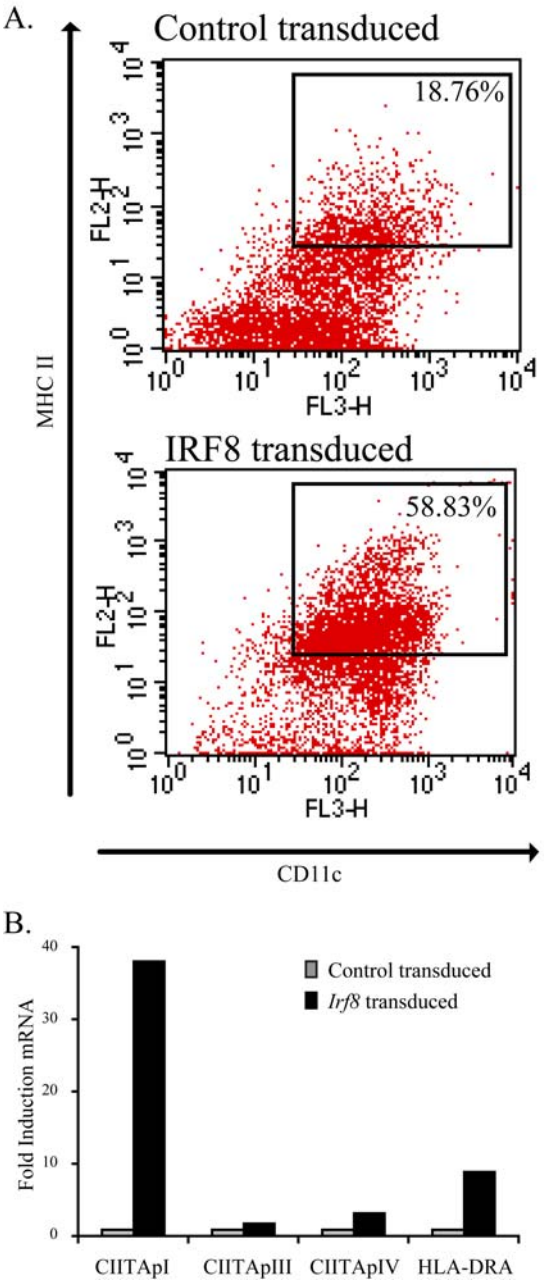
Supplemental Figure 2:



Supplemental Figure 3: Comparison of Human and Mouse CIITA Promoter I

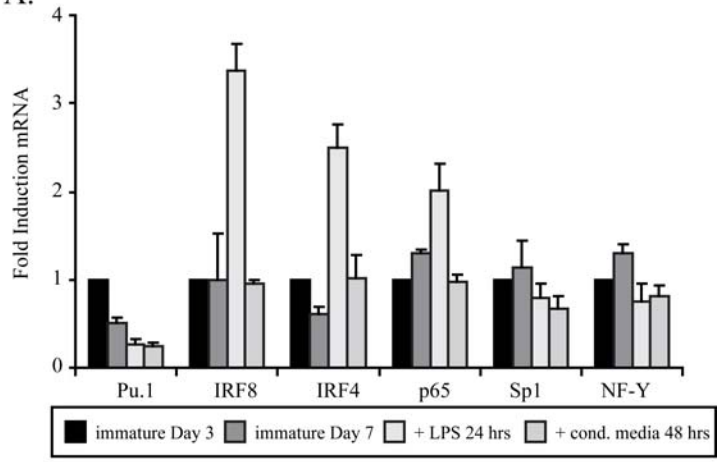


Supplemental Figure 4

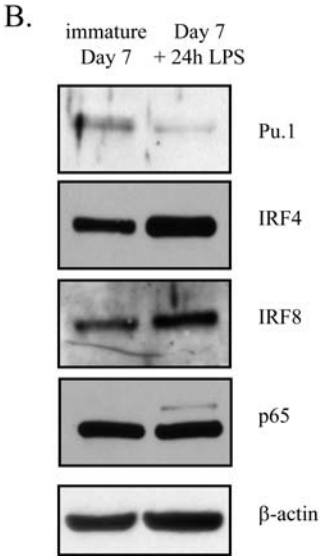


Supplemental Figure 5

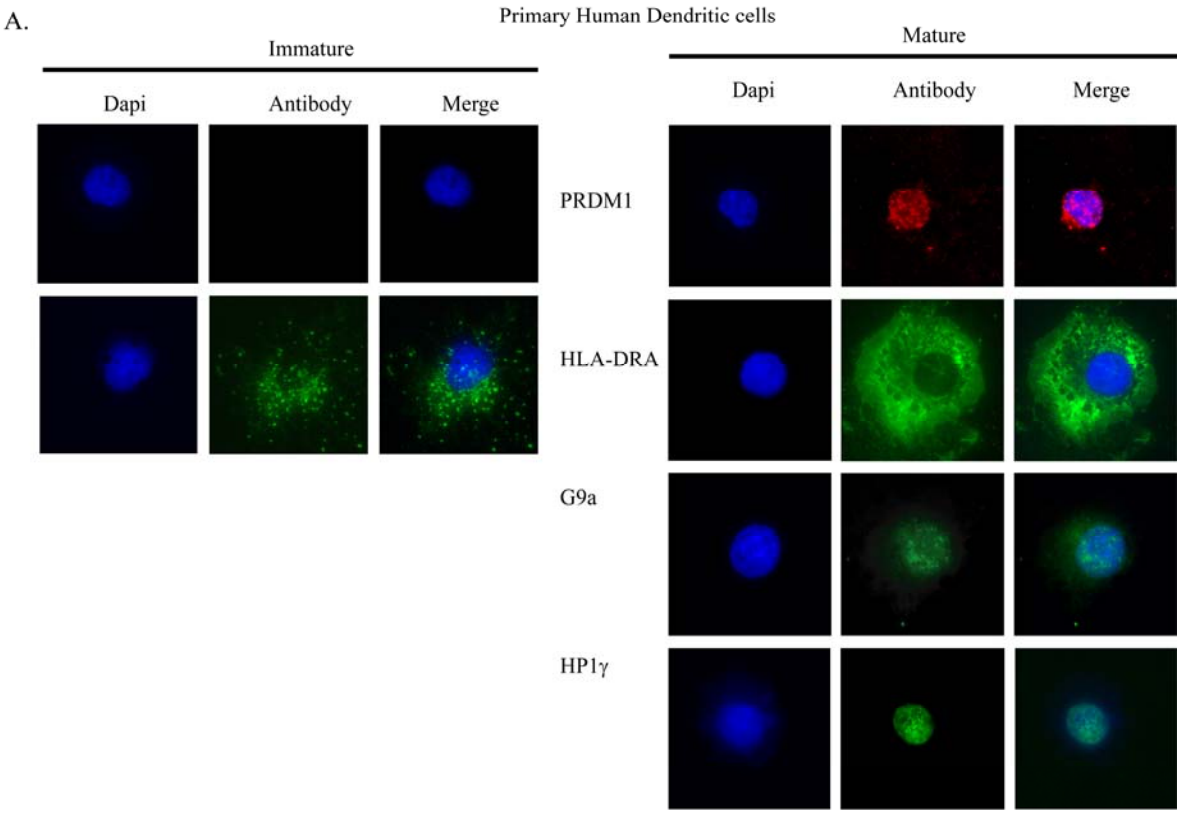
A.

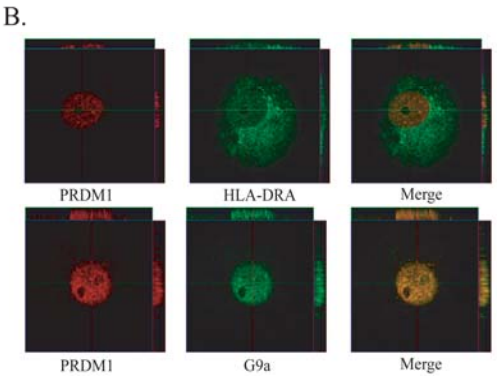


B.

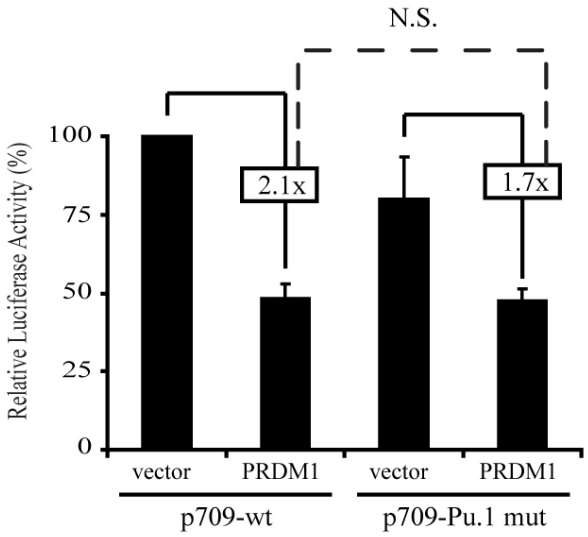


Supplemental Figure 6





Supplemental Figure 7



Supplemental Table 1

Human EMSA Probes

Name	Forward Primer 5'-3'	Reverse Primer 5'-3'
CIITAP1-Pu.1	GAT CGC AAA AGA GGA AAT CTT CCT AAG TAG AAA TAA	GAT CTT ATT TCT ACT TAG GAA GAT TTC CTC TTT TGC
CIITAP1-Pu.1 mutant 1	GAT CGC AAA AGA CAG AAT CTT CCT AAG TAG AAA TAA	GAT CTT ATT TCT ACT TAG GAA GAT TCT GTC TTT TGC
CIITAP1-Pu.1 mutant 2	GAT CGC AAA AGA GGA AAT CTC TGT AAG TAG AAA TAA	GAT CTT ATT TCT ACT TAC AGA GAT TTC CTC TTT TGC
CIITAP1-Pu.1 mutant 3	GAT CGC AAA AGA CAG AAT CTC TGT AAG TAG AAA TAA	GAT CTT ATT TCT ACT TAC AGA GAT TCT GTC TTT TGC
CIITAP1-EICE	CGT CCT GGT TTT CAC TTC ATG TTT TGG	GCA TCC AAA ACA TGA AGT GAA AAC CAG G
CIITAP1-EICE mutant 1	CGT CCT GGT TTT CAC TTA AGG TTT TGG	GCA TCC AAA ACC TTA AGT GAA AAC CAG G
CIITAP1-EICE mutant 2	CGT CCT GGT TAG TAC TTC ATG TTT TGG	GCA TCC AAA ACA TGA AGT ACT AAC CAG G
CIITAP1-EICE mutant 3	CGT CCT GCA GTT CAC TTC ATG TTT TGG	GCA TCC AAA ACA TGA AGT GAA CTG CAG G
CIITAP1-NF-kB-Sp-1	GAT GCT GCA TGC TGG GTG AGC GGA GAT TCC AGG CAC TG	GAT CAG TGC CTG GAA TCT CCG CTC ACC CAG CAT GCA GC
NFAT consensus oligo	GAT CCG CCC AAA GAG GAA AAT TTG TTT CAT A	GAT CTA TGA AAC AAA TTT TCC TCT TTG GGC G
C/EBPβ consensus oligo	GAT CTG CAG ATT GCG CAA TCT GCA G	GAT CCT GCA GAT TGC GCA ATC TGC A
PU.1 consensus oligo	GGG CTG CTT GAG GAA GTA TAA GAA T	ATT CTT ATA CTT CCT CAA GCA GCC C

Human qRT-PCR primers

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
CIITAP1	GGA GAC CTG GAT TTG GCC CT	GAA GCT CCA GGT AGC CAC CTT CTA
CIITAP3	CAA TGC GTT GCC TGG CTC	GCT GTT AAG AAG CTC CAG GTA G
CIITAP4	CAG AGC TGG CGG GAG GGA	GCT GTT AAG AAG CTC CAG GTA G
Total CIITA	CTC TGA GTG GCG AAA TCA AG	CAA TGC TAG GTA CTG CGG
HLA-DRA	GTC TGG CGG CTT GAA GAA T	ACC TTG AGC CTC AAA GCT G
PU.1	AGC AGA TGC ACG TCC TCG ATA	AGA CCT GGT GGC CAA GAC TG
ICSBP	ATG TGT GAC CGG AAT GGT G	CGG AAC ATG CTC TTC TTC TC
IRF-4	GTT TAA AGG AAA GTT CCG AGA AGG	TAC ACT TTG TAC GGG TCT GAG ATG
NF-kB p65	AAG CTG ATG TGC ACC GAC AA	CAT GGA GAC ACG CAC AGG AG
Sp-1	GCT GGC AGA TCA TCT CTT CTT	CCA GAG ACT GTG CGA TTC TTG
NF-YA	CTC TCT ACG TGA ATG CCA AAC AAT	CAT GCA GGT ATT TCC TTC TCT CCT
PRDI-BF1	TAC ATA CCA AAG GGC ACA CG	TGA AGC TCC CCT CTG GAA TA
GAPDH	GAA GGT GAA GGT CGG AGT	GAA GAT GGT GAT GGG ATT TC

Human qPCR primers for ChIP

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
hCIITAP1	GAA CCC CAG CCT ACA ACG	CTG GCC AGT GCC TGG AAT
hCIITAP3	CAG ACT TTC TGT GCA ACT TTC TGT	GAT TTC TGT TTC TGA ACA CCC TCT
hDRA	GAT CTC TTG TGT CCT GGA CCC TTT GCA AGA ACC CT	CCC AAT TAC TCT TTG GCC AAT CAG AAA AAT ATT TTG

Primers for In vivo Footprinting

Gene	Forward Primer 5'-3'
hCIITAP1 set Ap1	
hCIITAP1 set Ap2	GTT GTT CAT GGC AGC CCT TGG AGT CAG
hCIITAP1 set Ap3	TGT TCA TGG CAG CCC TTG GAG TCA GGG CA
hCIITAP1 set Cp1	TCT GTG GAG TCT GAA TCA AC
hCIITAP1 set Cp2	ATC AAC CCA AAA GCC AAT ATC CAT CCG
hCIITAP1 set Cp3	CCC AAA AGC CAA TAT CCA TCC GTT CAT CAG
mCIITAP1 set Ap1	AGA GCA GTG TCT GTA CTT GG
mCIITAP1 set Ap2	GGA AGT GGT TCA TGG CAG TGC TCT GAG
mCIITAP1 set Ap3	GAA GTG GTT CAT GGC AGT GCT CTG AGG CT
mCIITAP1 set Bp1	CCT TAC TCT TAT TGC TGT CC
mCIITAP1 set Bp2	GTC CAA GTC ACC CCT AAC CCA TTT CCG
mCIITAP1 set Bp3	CCC CTA ACC CAT TTC CGT TCA TCA GGC AC